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A review of the Amplified Fragment Length Polymorphism (AFLP) technique in genotyping and DNA fingerprinting studies

[Review Article]

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ABSTRACT
Amplified fragment length polymorphism (AFLP) is a marker based on polymerase chain reaction amplification of restricted fragments ligated to synthetic adaptors and amplified using primers which carry selective nucleotides at their 3' ends. The technique generates highly reproducible markers from DNA of any organism and allows high resolution genotyping. AFLP has broad applications and has been used to investigate genomes of different complexity from microbes to higher organisms for purposes of species, strains and varieties identification, systematics, pathotyping, population genetics, simple and complex trait mapping, population genetics, construction of linkage and physical maps. In addition, it is being used in medical diagnostics, forensic analysis and microbial typing. AFLP is superior compared to other markers in that it has time efficiency, generates more information, is highly reproducible and has a wide range of applications. The marker has a drawback in that it generates dominant rather than co-dominant markers and can also be expensive if automated systems are used.

Key words: DNA fingerprints, marker, polymorphism, restriction, sequence

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INTRODUCTION

A multitude of techniques are available for examining genetic variation between organisms by detecting polymorphisms in nuclear or organellar DNA (Rehner & Uecker, 1995; Madan *et al*., 1997; Uptmoor *et al*., 2003). Unlike protein markers, DNA markers are not subject to environmental influences and they exist in unlimited numbers covering the entire genome (Williams *et al*., 1990; Rita *et al*., 2002; Kinyua, 2004). Molecular markers have been used to detect genetic variation among a wide variety of organisms including microorganisms, insects, fish, humans, coral and plants (Sheriff *et al*., 1994; Brown, 1996; Otsen *et al*., 1996; Madan *et al*., 1997; Mueller & Wolfenbarger, 1999). The commonly used techniques include amplified fragment length polymorphism, restriction fragment length polymorphism, random amplified polymorphic DNA, single strand conformation polymorphism, sequence characterized amplified region, simple sequence repeats also known as microsatellites, simple sequence polymorphisms and sequence

tagged microsatellites (Freeman *et al*., 1993; Lin *et al*., 1996; McDonald, 1997; Majer *et al*., 1998; Rita *et al*., 2002; Wagara, 2004).

Molecular markers are based solely on the detection of naturally occurring DNA polymorphisms. These polymorphisms occur as a result of point mutations or rearrangements such as insertions or deletions in the DNA (Williams *et al*., 1990; Brown, 1996; Mueller & Wolfenbarger, 1999; Uptmoor *et al*., 2003; Kinyua, 2004). The polymorphism can be detected by scoring the presence or absence of bands in patterns that are generated either by restriction enzyme digestion or DNA amplification procedures or both (Otsen *et al*., 1996; Kiprop, 2001). Variations in banding patterns reflect the genetic relationship between organisms and therefore the patterns can be considered as genomic fingerprints that allow numerical analysis of the level of genetic diversity and phylogenetic relationships within and between species and to identify particular races and pathotypes (Freeman *et al*., 1993; Sheriff *et al*., 1994; Majer *et al*., 1998; Rehner & Uecker, 1995; Mueller & Wolfenbarger, 1999; Kinyua, 2004).

Some strains, biovars or races of plant pathogenic organisms are difficult to differentiate based on phenotypic/morphological features and hence require the use of molecular techniques (Freeman *et al*., 1993; Uptmoor *et al*., 2003). In fungi and other organisms, the non coding regions of the rDNA have been used as variable regions (Madan *et al*., 1997; McDonald, 1997). The internal transcribed spacers (ITS) of the rDNA can display variation within genera and can therefore be used to differentiate species (Rehner & Uecker, 1995). At intraspecific level, variability in the intergenic spacers sequence (IGS) that separates ribosomal repeat units is variable enough to allow discrimination of related fungi (Sheriff *et al*., 1994; Brown, 1996; Lin *et al*., 1996; Nichole *et al*., 1997; Rita *et al*., 2002).

AMPLIFIED FRAGMENT LENGTH POLYMORPHISM (AFLP)

The AFLP technique was developed in the early 1990s by Keygene, a company in Netherlands. It is a convenient, robust and reliable tool that has been used for genetic mapping/genotype identification, taxonomic and population genetic studies, medical diagnostics, in marker assisted selection, forensic analysis and microbial typing (Nichole *et al*., 1997; Majer *et al*., 1998; Savelkoul *et al*., 1999; Kiprop, 2001; Buhariwalla *et al*., 2005). AFLP has been used to study a wide variety of organisms including bacteria, nematodes, insects, fungi, plants, corals, humans and fish (Vos *et al*., 1995; Mueller *et al*., 1996; Mueller & Wolfenbarger, 1999; Terefework *et al*., 2001; Fanizza, *et al*., 2003). AFLP involves detection of the presence or absence of restriction fragments produced by restriction enzyme digestion of template DNA and two cycles of PCR (Otsen *et al*., 1996; Savelkoul *et al*., 1999; Terefework *et al*., 2001; Krauss, 2000; Kinyua, 2004).

In most cases, a few primer combinations are needed to generate an adequate number of polymorphic markers. To reveal differences between closely related or inbred individuals, AFLP markers have to be generated with a series of primer combinations (Nichole *et al*., 1997; Savelkoul *et al*., 1999; Terefework *et al*., 2001; Wagara, 2004). The power of AFLP is based upon the molecular genetic variations that exist between closely related species, varieties or cultivars. These variations in DNA sequences are exploited by the AFLP technology such that "finger prints" of particular genotypes can be routinely generated (Vos *et al*., 1995; Lin *et al*., 1996; Majer *et al*., 1998; Buhariwalla *et al*., 2005; Laurentin & Karlovsky, 2006).

Compared to other molecular markers the advantages of AFLP are that, it generates more information, is highly reproducible, and it has a wide range of applications. For example, it can be used for polymorphism screening (Steiger *et al*., 2002), quantitative trait loci analysis (Otsen *et al*., 1996), genetic mapping, identity, parentage and phylogenetic studies. No prior sequence data for primer construction is needed and hence there is no need for prior knowledge about the genomic make up of the organism (Vos *et al*., 1995). Furthermore, AFLP is not affected by small variations in PCR amplification parameters since these are performed under conditions of high selectivity/stringency (Krauss, 2000). AFLP method has few errors e.g. mispriming and scoring (less than 2%), the markers are highly abundant and distributed throughout the genome (Savelkoul *et al*., 1999), they

segregate in Mendelian fashion, it has time efficiency since it can be generated fast (Mueller *et al*., 1996; Wagara, 2004), and have a higher resolution revealing even minor genetic differences within any group of organisms. A very low amount of DNA is required which enables small organisms to be examined, e.g. DNA from single spores (Nichole *et al*., 1997; Mueller & Wolfenbarger, 1999; Kiprop, 2001; Uptmoor *et al*., 2003; Kinyua, 2004; Buhariwalla *et al*., 2005; Laurentin & Karlovsky, 2006).

The disadvantages of AFLP are that it requires pure and high molecular weight DNA (Vos *et al*., 1995) and alleles are not easily recognized since it is a dominant marker, thus allelic fragments are scored independently which can lead to an overestimation of variation (Otsen *et al*., 1996; Terefework *et al*., 2001; Steiger *et al*., 2002). This technique can also be expensive if automated systems are used. Loss of restriction sites, insertions or deletions may cause deviation from the true variation between individuals since fragment changes rather than site changes are scored (Lin *et al*., 1996; Mueller *et al*., 1996; Savelkoul *et al*., 1999; Krauss, 2000; Buhariwalla *et al*., 2005; Laurentin & Karlovsky, 2006). The 5 main steps of the AFLP technique are (1) digestion of the genomic DNA, (2) ligation, (3) pre-selective amplification, (4) selective amplification, (5) separation and visualization of the gel images (Nichole *et al*., 1997; Savelkoul *et al*., 1999; Uptmoor *et al*., 2003; Wagara, 2004).

Digestion / restriction of DNA: The genomic DNA is digested simultaneously with two restriction endonucleases consisting of a six base pair cutter and a four base cutter. Restriction endonucleases are protein enzymes that recognize specific nucleotide sequences and cleave both strands of DNA containing those sequences (Kiprop, 2001; Fanizza, *et al*., 2003; Buhariwalla *et al*., 2005).The amount of DNA used varies depending on the volume of the reaction mixture with approximately 500 ng being required for a 20 µl reaction mixture. The 4bp recognition sequence enzyme e.g. *Mse* I cuts frequently whereas the 6bp recognition sequence enzyme e.g. *Ecor* I or *Pst* I cuts less frequently since it is a rare cutter (Vos *et al*., 1995; Laurentin & Karlovsky, 2006). After cleavage, products with 5' or 3' overhanging single stranded ends may be produced (Mueller *et al*., 1996; Savelkoul *et al*., 1999; Krauss, 2000; Kinyua, 2004). Recognition sequences for many enzymes are the same on both strands. Such recognition sequences are said to be palindromic. The sites of cleavage of DNA are determined by their nucleotide sequence and the long DNAs are broken into discrete sized fragments (Nichole *et al*., 1997; Mueller & Wolfenbarger, 1999; Wagara, 2004).

The size of restriction fragment is determined by the distance between restriction enzyme cleavage sites. The frequency of cleavage of DNA depends on the probability of occurrence of a recognition sequence, thus enzymes with longer recognition sequence cuts less frequently and consequently produces larger fragments than those enzymes with shorter recognition sequences (Vos *et al*., 1995; Otsen *et al*., 1996; Savelkoul *et al*., 1999; Kiprop, 2001). The overhangs produced by many restriction enzymes are used as sticky ends to "glue" DNA fragments from different sources together. For AFLP, complete genomic restriction is necessary to prevent later amplification of uncut fragments (Nichole *et al*., 1997; Krauss, 2000; Steiger *et al*., 2002; Fanizza, *et al*., 2003; Buhariwalla *et al*., 2005). Complete digestion is achieved by the use of high quality DNA and an excess of restriction enzyme. Factors such as buffer composition, incubation temperature, DNA methylation and star activity due to digestion under non-standard conditions can hinder the activity of restriction endonucleases (Mueller *et al*., 1996; Savelkoul *et al*., 1999; Kinyua, 2004).

In some restriction reactions, the DNA may be digested simultaneously with the two restriction endonucleases. However, in a case where restriction enzymes require different buffer conditions, restriction is first performed with one enzyme (rare cutter) then the buffer composition is altered before adding the next enzyme for the second restriction (Vos *et al*., 1995; Krauss, 2000; Terefework *et al*., 2001; Laurentin & Karlovsky, 2006). In cases where two restriction enzymes have totally incompatible buffers, the digestion with the rare cutter is performed first and the DNA is then recovered (usually by precipitation) and re-suspended in the buffer appropriate for the second enzyme (Mueller *et al*., 1996; Fanizza, *et al*., 2003). Complete restriction of DNA is indicated by a smear after running restricted DNA on 0.8 % agarose gel (Steiger *et al*., 2002). Restriction digestion of genomic DNA generates the required substrate for ligation and subsequent amplifications (Otsen *et al*., 1996; Mueller & Wolfenbarger, 1999; Kiprop, 2001; Uptmoor *et al*., 2003). The restricted DNA can be stored at -20°C if ligation reactions are to be performed later.

Ligation reaction: The resulting restriction fragments are ligated to adapters, e.g. *Ecor* I and *Mse* I adapters to generate template DNA for amplification. The adapters are double stranded oligonucleotides consisting of a core sequence and an enzyme specific sequence (Steiger *et al*., 2002). These adapters are ligated to the ends of the restriction fragments (Savelkoul *et al*., 1999; Wagara, 2004). The ligation of the adapters to restricted DNA alters the restriction site so as to prevent a second restriction from taking place after ligation has occurred (Krauss, 2000). Adaptor ligations can be performed in the presence of restriction enzymes such that any fragment to fragment ligations are immediately re-cleaved by the restriction enzyme (Vos *et al*., 1995; Otsen *et al*., 1996; Steiger *et al*., 2002; Kinyua, 2004).

The end sequences of each adapted fragment contains the adaptor sequence and the remaining part of the restriction sequence (Mueller & Wolfenbarger, 1999; Fanizza, *et al*., 2003). These known end sequences serve as priming sites in the subsequent AFLP-PCR (Kiprop, 2001). Ligation reaction is performed by adding T_4 DNA ligase into the restricted DNA together with the adapter ligase solution and incubating for 2 hours at 20°C. After the ligation, the DNA ligase enzyme is deactivated by incubating at 65°C for 10 minutes followed by a 1:10 dilution with T.E. buffer (Nichole *et al*., 1997; Krauss, 2000; Uptmoor *et al*., 2003; Laurentin & Karlovsky 2006). The diluted and ligated mixture can then be used for pe-amplification or stored at -20°C until further use.

Pre-selective amplification: Depending on genome size, restriction ligation generates thousands of adapted fragments. Only a subset of these fragments needs to be amplified and pre-selective amplification is done to reduce the complexity of the adapted fragment population (Savelkoul *et al*., 1999; Krauss, 2000; Kiprop, 2001). This is done with primers complimentary to the adaptor sequences, each with one selective nucleotide. The pre-selective PCR amplification is performed under highly stringent conditions to ensure that only perfect matches are primed and elongated (Mueller *et al*., 1996; Nichole *et al*., 1997; Steiger *et al*., 2002; Fanizza *et al*., 2003; Laurentin & Karlovsky, 2006). A primer extension by one nucleotide reduces the number of amplified fragments by a factor of 4. Because of the high selectivity, primers differing by only a single base pair in the AFLP extension amplify different subsets of fragments (Vos *et al*., 1995; Wagara, 2004). After the pre-selective amplification, a 1:10 or 1:50 dilution of the PCR products is done using TE buffer. If the previous steps have worked well, a clear DNA band is seen on running the PCR products on 1.2% agarose gel (Mueller & Wolfenbarger, 1999; Buhariwalla *et al*., 2005).

Selective amplification: Selective amplification is basically similar to the pre-selective amplification, except that the primers used have 2 - 3 selective nucleotides. The number of amplified fragments is further reduced since only subsets of fragments having matching nucleotides at all the three positions are amplified, which further reduces the complexity of PCR products mixture (Otsen *et al*., 1996; Mueller & Wolfenbarger, 1999; Terefework *et al*., 2001; Kinyua, 2004). A primer extension of two or three base pairs reduces the number of amplified fragments by a factor of 16 and 64, respectively. Up to 4 selective nucleotides can be included in the selective amplification and the ideal extension lengths vary with the genome size (Krauss, 2000; Steiger *et al*., 2002). By using combinations of primers with different extensions, a series of AFLP amplifications can thus screen a representative fraction of the genome (Mueller & Wolfenbarger, 1999; Fanizza, *et al*., 2003).

The ideal number of bands generated by AFLP should range between 50 – 100 since too many bands cause smears (Vos *et al*., 1995). The number of bands depends on the selective nucleotides in the primers and the complexity of the genome (Wagara, 2004; Laurentin & Karlovsky, 2006). When using capillary systems in scoring, the complexity of the banding pattern can be reduced by radioactively labeling the primer directed against the rare cutter adaptor e.g. *Ecor* I adaptor sequence, since *Mse* I – *Ecor* I and *Ecor* I – *Ecor* I fragments are a more limiting subset of the total DNA fragments (Mueller *et al*., 1996; Reineke & Karlovsky, 2000, Papa *et al*., 2005). After selective amplification, formamide dye is added to the PCR products followed by denaturation for 2 – 3 minutes at 85°C and the denatured products are immediately placed on ice (Savelkoul *et al*., 1999; Kiprop, 2001; Uptmoor *et al*., 2003).

Scoring AFLP markers: AFLP – PCR products can be separated and scored using a variety of techniques ranging from simple gel electrophoresis to automated genotyping. Agarose gel electrophoresis, though cheap and user friendly, gives the least resolution and is unable to score fragment length differences of less than 10 nucleotides (Vos *et al*., 1995; Savelkoul *et al*., 1999; Kinyua, 2004). Polyacrylamide gel electrophoresis, done either manually or with automated sequence provides the maximum resolution of AFLP banding patterns to the level of single nucleotide differences (Sheriff *et al*., 1994; Nichole *et al*., 1997; Mueller & Wolfenbarger, 1999; Kiprop, 2001; Terefework *et al*., 2001; Fanizza *et al*., 2003; Brugmans *et al*., 2003; Papa *et al*., 2005). Capillary sequencers can also be used that register signals in electropherograms which are analysed by computer software thus eliminating the need for manual scoring and analysis (Otsen *et al*., 1996; Mueller *et al*., 1996; Steiger *et al*., 2002; Buhariwalla *et al*., 2005). Compared to other methods, the use of capillary systems and automated analysis increases data throughput and scoring reliability, thus decreasing the overall experimental error (Papa *et al*., 2004).

Analysis of AFLP fingerprints: Bands are assigned numbers in relation to their migration distance within the gel. Bands with the highest molecular weight are assigned number one and so on until the band with the lowest molecular weight (Excoffier *et al*., 1992; Savelkoul *et al*., 1999). It is assumed that bands of the same molecular weight in different individuals are identical in sequence (Wagara, 2004). Presence or absence of a band is given a score of 1 or 0, respectively (Steiger *et al*., 2002; Brugmans *et al*., 2003). Similarity matrices from binary banding data are derived with appropriate software, e.g. NTSYSpc-Numerical Taxonomy and multivariate analysis system for personal computer (Saitou & Nei, 1987; Rohlf 1998; Kiprop, 2001; Steiger *et al*., 2002; Laurentin & Karlovsky 2006). Estimates of similarity are done using coefficients such as Jaccard and matrices of similarity analysed using the unweighted pair group method with arithmetic averages (UPGMA) clustering method (Sheriff *et al*., 1994; Mueller *et al*., 1996; Fanizza, *et al*., 2003). Dendograms, principle coordinate analysis and genetic distance matrices can be generated using appropriate software to help in interpreting the genetic relatedness of the organisms or strains being studied (Saitou & Nei, 1987; Excoffier *et al*., 1992; Vos *et al*., 1995; Savelkoul *et al*., 1999; Terefework *et al*., 2001; Brugmans *et al*., 2003; Wagara, 2004; Buhariwalla *et al*., 2005; Papa *et al*., 2005).

Conclusion

The reliability of AFLP coupled with its wide applications makes it a useful marker in pathotyping, systematics, mapping of quantitative trait loci, population genetic studies and in differentiating closely related organisms. Due to its high sensitivity to minor genetic variations, AFLP will remain a key molecular tool, even replacing several other techniques such as RFLP and microsatellites. However, due to its high cost and dominant outcomes, the decision to use AFLP should be made based on the research question being addressed and availability of facilities. Although other codominant markers such as microsatellites allow more powerful population genetic analysis, AFLP is more reliable and easier to use and thus will remain a popular marker in DNA fingerprinting studies.

References

Brown JKM, 1996. The choice of molecular marker methods for population genetic studies of plant pathogens. New Phytol 133:183 - 195.

Brugmans B, van der Hulst RGM, Visser RGF, Lindhout P, van Eck HJ, 2003. A new and versatile method for the successful conversion of AFLP markers into simple single locus markers. Nucleic Acids Res 31(10):e55.

Buhariwalla HK, Srilakshmi, P, Kannan S, Kanchi RS, Chandra S, Satyaprasad K, Waliyar F, Thakur RP, Crouch JH, 2005. AFLP analysis of Trichoderma spp. From India compared with sequence and morphological-based diagnostics. Journal of Phytopathology 153: 389-400.

Excoffier L, Smouse P, Quattro J, 1992. Analysis of molecular variance inferred from metric distance among DNA haplotypes; application to human mitochondrial DNA restriction data. Genetics 131:479-491.

Fanizza G, Chaabane R, Lamaj F, Ricciardi L, Resta P, 2003. AFLP analysis of genetic relationships among aromatic grapevine (*Vitis vinifera*). Theor Appl Genet, 107:1043-1047.

Freeman S, Pharm M, Rodriguez R, 1993. Molecular genotyping of *Colletotrichum* species based on arbitrarily primed PCR, A+ T-rich DNA analyses. Exp. Mycol. 17: 309 - 322.

Kinyua ZM, 2004. Genetic structure, virulence characteristics and survival of *Cercospora* populations causing maize grey leaf spot in Kenya. PhD Thesis. Royal Holloway University of London.

Kiprop EK, 2001. Characterization of *Fusarium udum* Butler isolates and wilt resistance pigeon pea in Kenya. Ph D. Thesis, University of Nairobi. 218pp.

Krauss S, 2000. Accurate gene diversity estimates from amplified fragment length polymorphism (AFLP) markers. Molecular Ecology 9:1241-1245.

Laurentin EH. and Karlovsky P, 2006. Genetic relationship and diversity in a sesame (*Sesamum indicum* L.) germplasm collection using amplified fragment length polymorphism (AFLP) BMC. Genetics 7:10.

Lin JJ, Kuo j, Jin M, Saunders JA, Beard SH, McDonald H, Kenworth W, Ude GN, Mathews BF, 1996. Identification of molecular markers in Soybean; comparing RFLP, RAPD and AFLP DNA mapping techniques. Plant Molecular Biology Reporter 14:156-169.

Madan M, Nair S, Bhagwat A, Krishna TG, Masohiro Y, Bhatia CR, Sasaki T, 1997. Genome mapping, molecular markers and marker assisted selection in crop plants. Molecular Breeding 3: 87-103.

Majer D, Lewis BG, Mithen R, 1998. Genetic variation among field isolates of *Pyrenopeziza brassicae*. Plant Pathol. 47: 22-28.

McDonald BA, 1997. The population genetics of fungi: Tools and Techniques. Phytopathology 87: 4448 - 4453.

Mueller GU. and Wolfenbarger LL, 1999. AFLP genotyping and fingerprinting. TREE 14: 389-394.

Mueller UG, Lipari SE, Milgroom MG, 1996. Amplified fragment length polymorphism (AFLP) fingerprinting of symbiotic fungi cultured by the fungus growing ant *Cyphomyrmex minutes*. Mol. Ecol. 5: 119-122.

Nichole RO, Berkum P, Lin JJ, Kuo J, Ude GN, Kenworthy W, Saunders JA, 1997. Application of amplified fragment length polymorphism for genetic characterization of Colletotrichum pathogens of alfafa. Phytopathology 87: 745 - 750.

Otsen M, Bieman M, Kuiper MT, Pravenec M, Kren V, Kurtz TW, Jacob HJ, Lankhorst A, Van Zutphen BF, 1996 Use of AFLP markers for gene mapping and QTL detection in the rat. Genomics 37: 289 - 294.

Papa R, Troggio M, Ajmone-Marsan P, Nonnis Mar F, 2005. An improved protocol for the production of AFLPTM markers in complex genomes by means of capillary electrophoresis J. Anim. Breed. Genet. 122: 62 - 68.

Rehner SA. and Uecker FA, 1995. Nuclear ribosomal internal transcribed spacer phylogeny and host diversity in the coelomycete *Phomopsis*. Can. J. Bot. 72:1666 -1674.

Reineke A. and Karlovsky P, 2000. Simplified AFLP protocol: replacement of primer labeling by the incorporation of alpha-labeled nucleotides during PCR. Bio Techniques 28: 622-623.

Rita CB, Aparecid DR, Henrique MV, Rachael BC, Edilson P, 2002. Genetic variation among pathogens causing "Helminthosporium" diseases of rice, maize and wheat. Sete Lagoas. Brazil.

Rohlf FJ, 1998. NTSYS-pc. Numerical Taxonomy and Multivariate Analysis System, Version 2.0. New York, USA, Applied Biostatistics.

Saitou N. and Nei M, 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425.

Savelkoul P, Aarts H, DeHaas J, Dijkshoorn L, Duim B, Otsen M, Rademaker J, Schouls L, Lenstra J, 1999. Amplified-fragment length polymorphism analysis: the state of an art. Journal of Clinical Microbiology 37: 3083 - 3091.

Sherriff C, Whelan, MJ, Arnold GM, Lafay JF, Brygoo Y, Bailey JA, 1994. Ribosomal DNA sequence analysis reveals new species groupings in the genus *Colletotrichum*. Exp. Mycol. 18: 121-138.

Steiger L, Nagai C, Moore H, Morden W, Osgood V, Ming R, 2002. AFLP analysis of genetic diversity within and among *Coffea arabica* cultivars. Theor Appl Genet 105: 209-215.

Terefework Z, Kaijalainen S, Lindstrom K, 2001. AFLP fingerprinting as a tool to study the genetic

diversity of *Rhizobium galegae* isolated from *Galega orientalis* and *Galega officinalis*. J. Biotechnol. 91: 169 - 180.

Uptmoor R, Wenzel W, Friedt W, Donaldson G, Ayisi K, Ordon F, 2003. Comparative analysis on the genetic relatedness of Sorghum bicolor accessions from Southern Africa by RAPDs, AFLPs and SSRs. Theor Appl Genet. 106: 1316-1325.

Vos P, Hogers R, Bleeter M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M, 1995. AFLP: a new concept for DNA fingerprinting. Nucleic Acids Res. 23: 4407- 4414.

Wagara IN, 2004. Molecular and virulence characterization of *Phaeoisariopsis griseola* and reaction of bean germplasm to races of the angular leaf spot pathogen. PhD. Thesis, University of Nairobi. 169pp.

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey S, 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18: 6531 - 6535.